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PASSIVE IMMUNITY EVALUATION IN PIGLETS ORIGINATING FROM SOWS VACCINATED WITH CHINA STRAIN OF CLASSICAL SWINE FEVER VIRUS

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An experimental study was conducted to investigate the course of classical swine fever (CSF) infection in piglets originating from sows vaccinated with China strain vaccine. The experiment was carried out on 24 piglets (age 28, 35, 44 and 54 days) from vaccinated sows and on 11 non vaccinated piglets, originated from non CSF vaccinated sows. Two piglets from the each age group originating from vaccinated sows were challenged by intramuscular injection with CSF virus. Four piglets of the same age from vaccinated sows, and two piglets derived from unvaccinated sows were added to the challenged group to determine contact (horizontal) infection. After challenge, clinical examination and blood sampling from every animal was carried out on day 0, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27 and 29. Blood samples were examined for CSF virus specific antibodies by ELISA test, and for viral antigen i. e. viral RNA by RT-PCR techique. After death or sacrifice pathomorphological changes, presence and distribution of CSF virus antigen were detected in piglet tissue samples by ELISA test. On the basis of the obtained results it can be concluded that not all piglets born to vaccinated sows have maternal antibodies at a detectable level, and the issue of the efficiency of passive immunization needs to be evaluated in the future.

Key words: classical swine fever, maternal antibodies, passive immunity, RT-PCR

INTRODUCTION

Classical swine fever (CSF) is an important viral disease caused by an RNA-virus, belonging to the family *Flaviviridae*, genus *Pestivirus* (Thiel *et al.,* 1996). The control of CSF in the European Union (EU) has been based on a policy of non-vaccination and stamping-out since 1980 (Dewulf, 2002). In spite of the eradication program implemented within the EU, outbreaks of the disease continue to occur, leading to serious losses (Moennig, 2000). In countries, in which CSF is endemic, prevention and control depend primarily on vaccination programs, using attenuated live-virus vaccines (Edwards *et al.,* 2000).

Extensive vaccination with viral vaccines creates a problem, because early vaccination of young animals frequently fails to establish solid immunity due to the interference of colostral antibodies upon vaccination. A particular problem represents the efficiency and the protective value of maternal antibodies in piglets that originate from repeatedly vaccinated sows (Lai *et al.,*1980). According to Terpstra (1988), offspring from vaccinated sows is protected to a large extent against lethal infection during the early days of life, but not against multiplication and excretion of the virus. Such piglets may experience a subclinical infection even with a strain of high virulence.

The objective of this trial was to examine whether the experimental infection of piglets aged 28, 35, 44 and 54 days, originating from sows vaccinated with China (C) - strain of classical swine fever virus (CSFV), will result in clinically evident or unapparent course of the disease. The aim of the research was also to investigate whether the infected piglets excrete CSFV sufficiently to infect susceptible pigs held in close contact. Emphasis was put on detection of CSFV antigen and genome in blood and tissue samples of piglets during 30 days after challenge.

MATERIAL AND METHODS

Animals and virus

The experiment was carried out on 35 clinically healthy, conventional weaner pigs, divided in 5 groups (group A, B, C, D and E). The experimental groups A, B, C and D consisted of 6 piglets of different age, i.e. 21, 28, 37 and 47 days, respectively. Experimental animals from these groups originated from the sows that were vaccinated several times with C-strain of CSFV. The experimental group E consisted of 11 susceptible pigs (not vaccinated against CSF, originating from unvaccinated sows), 35-40 days old. All experimental animals were examined for the absence of bovine viral diarrhoea virus (BVDV-1 and BVDV-2) antibodies and CSFV antigen at their arrival. After selecting the piglets ear tagging was carried out. For the challenge CSFV (strain Baker) was used. The isolate was verified to be free from BVDV by means of RT-PCR. The pigs were challenged with a dose of 1 mL by intra-muscular (i/m) route. The titre was 2×10^5 median tissue-culture-infective doses ($TCID_{50}$/mL).

Experimental design

The experiment was divided in three subsequent periods: the acclimatization, the challenge and the post-challenge period. Upon arrival, animals were allocated systematically to pens within 5 separate compartments. After an acclimatization period of 7 days, when the piglets originating from vaccinated sows were 28, 35, 44 and 54 days old, two piglets from each group were randomly selected, separated and than i/m inoculated with CSFV. To perform the inoculation the selected pigs were moved to separate compartments, where they remained 7 hours after challenge. Afterwards, i/m challenged pigs were reintroduced into their respective pens, in close contact with the other

piglets of the same age group. On the same day, 2 susceptible animals from group E were included into each of the above mentioned age groups, with the aim to investigate the possibility of horizontal (contact) infection. The control group consisted of 3 remaining piglets from group E, where experimental infection was done on the same day, according to the same model (i/m, 1 mL). All groups of piglets were housed under identical conditions in five separate compartments. Each compartment consisted of a well isolated area of 35 m^2, with independent ventilation, assigned for experimental work with animals. Technical design and biosafety measures entirely prevented the possibility of mechanic transmission of the virus from one compartment into the other. All materials necessary for blood sampling, rectal temperature monitoring, cleansing of the compartments and pens, and feeding of the pigs were provided per compartment and were stored at the compartment. After the challenge, each time before sample collection, all the pigs were clinically examined. The following symptoms were recorded during clinical examination: liveliness (apathy, lethargy), conjunctivitis, constipation-diarrhoea, coughing, ataxia, convulsions, posterior paresis, erythema and haemorrhages of the skin. Rectal temperature and mortality were recorded daily.

Sample collection - blood, sera and tissue samples

From the day when 2 piglets from groups A, B, C, D and 3 piglets from group E were experimentally infected, sera and heparinized blood samples were collected from the jugular vein of all piglets on 0, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27 and 29 days post-infection (dpi). During the experiment 256 samples of unclotted blood and 120 blood sera samples were taken.

For CSF serum antibody detection the commercial indirect immunoenzyme test (ELISA) kit (Herd Check CSFV Ab-ELISA test; IDEXX Scandinavia, Osterbybruk, Sweden) was used according to the manufacturer's instruction. For establishing the presence of BVDV antibodies virus-neutralization (VN) test was used. The test was carried out using 100 $TCID_{50}$/0.1 mL NADL (BVDV-1) and 178003 (BVDV-2) strains of BVDV, on BT cell culture (previously proved as BVDV free). For CSFV antigen detection in heparinized blood and tissue samples the commercial direct E^{rns} ELISA test kit (Herd Check CSFV Ag ELISA Test Kit; IDEXX Laboratories, Scandinavia, Osterbybruk, Sweden) was used according to the manufacturer's instructions.

Necropsy was performed on all animals immediately after death. Surviving piglets were euthanized on 35 dpi (T61®, Intervet International). Tissue samples (tonsils, spleen, kidney, terminal part of ileum and mandibular lymph nodes) were collected from every piglet in order to examine the presence and distribution of CSFV antigen. The total number of tissue samples was 175.

Detection of viral RNA in blood and tissues samples

Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) test was applied to detect genomic RNA of CSFV in heparinized blood and tissue samples. This test was used for the direct detection of CSFV in blood and tissue samples that gave negative or doubtful result on CSFV antigen in ELISA. A total of 52 blood and 20 tissue samples were examined by RT-PCR test. RNA was extracted from

blood and tissues using TRIzol reagent ("Gibco BRL", "Invitrogen Life Technologies", UK) according to manufacturer's recommendations. Briefly, 750 μL of TRIzol reagent was mixed with 250 μL of sample material (blood or supernatant from 10% tissue homogenate in PBS). After 10 min 200 μL of chloroform ("SIGMA", USA) was added, mixed and the suspension was centrifuged (Centrifuge 5415R, "Eppendorf", Germany) for 15 min at 14.000 g. The RNA containing aqueous phase was precipitated with 500 μL of isopropanol ("SIGMA", USA), maintained at room temperature for 10 min, and centrifuged for 10 min at 14.000 g. The RNA pellet was washed with 500 μL of 75% ethanol, centrifuged for 5 min at 12.000 g, dried, and resuspended in 40 μL of diethyl pyrocarbonate (DEPC) treated water (Promega Corporation, UK).

The "one-tube" or "one-step RT-PCR" assay was performed by using reagents supplied in a commercial "Access RT-PCR system", (Promega Corporation, UK) according to the manufacturer's instruction. RT-PCR amplification was done using E2 gene specific primers described by Katz *et al.* (1993) and manufactured by "LKB Vertriebs GmbH", Austria: gp55-U: 5'-ATA TAT GCT CAA GGG CGA GT-3' (sense, position in genome of the Alfort strain is 3378-3397); gp55-L: 5'-ACA GCA GTA GTA TCC ATT TCT TTA-3' (antisense, position in genome of the Alfort strain is 3685-3662). A 6 μL of RNA sample was added to a 44 μL reaction mixture containing AMV/*Tfl* 1 x reaction buffer, dNTP mix (10 mM), 25 pmol of sense and antisense primer, 1 mM of $MgSO_4$, 5 U of AMV RT and 5 U of *Tfl* DNA polymerase. The RT-PCR cycling conditions were as follows, 45 min at 48°C for RT, 94°C for 2 min for AMV RT inactivation and RNA/cDNA/ primer danaturation, 40 cycles of 30 s at 94^0C, 1 min at 60^0C and 2 min at 68^0C, and a final extension step at 68^0C for 7 min, and were performed in Mastercycler gradient thermocycler ("Eppendorf", Germany). After electrophoresis (Hoefer HE 33 mini submarine, "Amersham Biosciences") on a 1.5% agarose gel, amplified products were analysed on "Vilber Lourmat" France TFX-35.M transilumanator.

RESULTS AND DISCUSSION

Detection of CSFV antibodies

In pre-challenge sera samples from piglets 28, 35 and 54 days old, in each age group only in 50% of the examined samples maternally derived antibodies (MDA) were detected (Table 1). The smallest number of MDA positive sera samples were detected in piglets at 44 days of age, i.e. MDA was detected in one piglet only (No. III/5). According to records data, sows whose piglets were used in our trial had been vaccinated with C-strain at least 5 times in their lifetime.

Maternal antibodies ingested through colostrum protect young piglets against mortality due to CSF (Terzić *et al.,* 1998). This protection declines as piglets grow older, and maternal antibody titres decrease (*Terpstra and Wensvoort,* 1987). It is assumed that passive immunity is primarily dependent on the antibody titre of the mother and on the amount of colostrum ingested by the newborn. The rate of antibody decline was constant within litters and varied significantly between litters (Müller *et al.,* 2005). Terpstra and Wensvoort (1987)

Table 1. Results of experimental blood sera examined by ELISA test on presence of CSFV antibodies

Group A	No.	Days post infection										
		0	11	13	15	17	19	21	23	25	27	29
i/m challenge	I/1	+	†									
	I/6	–	†									
Contactly infected piglets	I/2	+	–	–								
	I/3	±	–	–	–	–	–	–	–	–	–	±
	I/4	+	–	–	†							
	I/5	±	†									
Susceptible piglets	N/1	–	–	†								
	N/2	–	–	†								
Group B												
i/m challenge	II/2	–	†									
	II/3	+	†									
Contactly infected	II/1	+	–	–	†							
	II/4	+	–	†								
	II/5	–	†									
	II/6	±	–	†								
Susceptible piglets	N/3	–	–	–	†							
	N/4	–	–	–	†							
Group C												
i/m challenge	III/3	–	†									
	III/4	–	†									
Contactly infected	III/1	±	–	†								
	III/2	–	†									
	III/5	+	–	–	–	–	–	–	†			
	III/6	±	–	†								
Susceptible piglets	N/5	–	–	–	–	†						
	N/6	–	–	–	–	–	†					
Group D												
i/m challenge	IV/3	–	†									
	IV/5	+	+	+	+	+	+	+	+	+	+	+
Contactly infected	IV/1	–	–	–	–	–	–	†				
	IV/2	–	+	+	+	+	+	+	+	+	+	+
	IV/4	+	+	+	+	+	+	+	+	+	+	+
	IV/6	–	–	–	–	–	†					
Susceptible piglets	N/7	–	–	+	+	+	†					
	N/8	–	–	–	†							
Group E												
Susceptible piglets	K/1	–	–	†								
	K/2	–	–	†								
	K/3	–	†									

No. – ear-tag number of pigs; (–) negative ELISA result; (±) doubtful ELISA result; (+) positive ELISA result; † – death of experimental animal

discovered that following re-vaccination of 5-7 months old gilts, animals with higher initial titres (titre prior to vaccination) generally showed a decrease of the titre. The results of present investigation suggest that changes of antibody in pregnant sows should be investigated in the future.

In i/m challenged piglet (No. IV/5) and in two piglets 54 days of age that survived contact infection (No. IV/2, IV/4), in which colostral antibodies were detected, in all the examined sera samples (from 11-29 dpi) the presence of CSFV antibodies was detected. On the contrary, in the pre-challenge sera sample from piglet 28 days old (No. I/3) that survived horizontal infection, doubtful result on the presence of MDA by ELISA test were detected. This finding is in agreement with the opinion that there is a "grey" zone of antibody titres, in which the outcome of a challenge is unpredictable (Terpstra and Wensvoort, 1987). The results of our experiment suggest that not all piglets born to vaccinated sows have MDA at a detectable level and this emerges the question of the efficiency of passive immunity. Serological finding from a piglet that originated from an unvaccinated sow and was exposed to contact infection (No. N/7), should be underlined since CSFV antibodies were detected from 13 dpi until death. Similar results are reported by Dewulf (2002) and Milanov *et al.* (2002), i.e. after challenge they detected the first CSFV antibodies from 11 and 13 dpi. Immune response after infection with CSFV is mostly determined by the appearance of neutralizing antibodies, and most often may be found in pigs that survived the infection with the highly virulent CSFV (Terpstra, 1988). It is assumed that neutralizing antibodies against CSF are detectable 2 weeks after infection at the earliest (Moennig, 2000).

Clinical signs of the disease

Piglets originationg from vaccinated sows i/m infected with CSFV

The i/m infected piglets become febrile on 3 dpi, exept the piglets 44 days of age, who showed increased body temperature already on 2 dpi. After that, in all piglets clinical signs compatible with CSF were noticed (apathy, dullness, conjunctivitis, diarrhoea). Locomotive disturbances and ataxia were noticed for the first time on days 5-7 dpi, and signs of posterior paresis on 7-8 dpi until death. All piglets died until 11 dpi. However, in one animal at 54 days of age (No. IV/5), only in the period from 3-6 dpi clinical signs of the disease were noticed: reduced appetite, dullness, reluctance to move. From 6 dpi until the end of the trial, there were no clinical signs of the disease. This piglet survived the experimental infection and was euthanized on day 35 dpi.

The established body temperatures and clinical signs correspond to characteristic signs of acute disease (Dahle and Liess, 1992; van Oirschot and Terpstra,1989). All the piglets were challenged with the same virulent strain of CSFV at the dose that can overcome passive immunity. Since these piglets were of the same nutritive status and from the herd on which continually is carried out an immunoprophylaxis program against CSF, it can be suggested that the consequence that influenced the outcome of the infection was associated with individual characteristics of the animal (passive immunity).

Clinical signs of disease and death of piglets of the same age that originated from vaccinated sows and the susceptible piglets can be considered sound evidence that i/m challenged piglets excreted a sufficient amount of CSFV to provoke contact infection. It is assumed that the principal mean of CSFV transmission is direct contact between infected and susceptible pigs (Terpstra, 1988), and under natural conditions the main portal of CSFV infection in the pig is the oronasal route. Infected pigs may shed the virus before the onset of disease and continue to do so during the entire disease period (Terpstra, 1988). It is assumed that it takes on average 4 days for a challenged pig to become infected (Dewulf, 2002), although initial viraemia occurs approximately 16-24 hours after infection (Trautwein, 1988).

Piglets infected by contact originating from vaccinated sows

In horizontally infected piglets originating from vaccinated sows, fever was recorded for the first time on 11, 13 and 14 dpi. After that, in all piglets clinical signs compatible with CSF were noticed. There were no differences in clinical symptoms of the disease depending on the age of the piglets. Constipation was recorded in most of the animals, and was followed by diarrhoea that persisted until death. Locomotive disturbance and ataxia of severe intensity were observed in piglets at the age of 35 days, while signs of posterior paresis appeared in most of the animals starting from day 12-13 dpi until death. The piglets died until 23 dpi, exept 3 piglets, who showed different clinical symptoms. One piglet age 28 days (No. I/3), starting from 23 dpi, exhibited exanthema on the skin of the pelvic limbs, but clinical signs were generally of weak intensity, whereby the symptoms of respiratory disease dominated (dispnea, caughing, purulent nasal discarge). This piglet survived contact infection and on 35 dpi was euthanised. Kleiboeker (2002) assumes that in cases when CSF occurs in vaccinated herds, the disease has a chronic course with a non-characteristic clinical picture. Two piglets age 54 days (No. IV/2 and IV/4) did not show signs of disease, nor increase of body temperature and on 35 dpi they were euthanized. Similar findings were reported in literature, but for adult pigs, where animals can recover with concurrent appearance of detectable neutralizing antibodies (Moennig, 2000).

Susceptible piglets infected via contact

In this subgroup pyrexia started on 6, 8, 9 and 10 dpi. The recorded clinical signs included inappetence, apathy, conjunctivitis and constipation. Skin lesions were recorded in the following sequence: cyanosis on 11-12 dpi, erythema on 13-14 dpi and petechial haemorrhagies from 16 dpi. The terminal stage was characterized by muscle tremor, convulsions and posterior paresis. All the animals in this group died from 14 to 20 dpi.

Control group

Following CSFV challenge, all animals exhibited distinct clinical signs of CSFV infection starting from 2 and 3 dpi. Clinical symptoms included fever, lethargy, conjunctivitis, constipation and starting from 4 dpi, diarrhoea. The

clinical picture was dominated by locomotory disorders with signs of ataxia and signs of posterior paresis. All the animals in this group died on 13 dpi.

In susceptible piglets of both groups clinical sigs were characteristic for acute CSF infection (Dahle and Liess, 1992), but were more severe comparing with signs in piglets that originate from vaccinated sows. Certainly in these piglets the virulence of the virus had the greatest influence on the course of the disease. Host factors seem to be of minor importance on the outcome of infections with a highly virulent CSF strain (van Oirschot and Terpstra, 1989), as it is a case of unvaccinated piglets, originating from unvaccinated sows (susceptible pigs).

Gross morphological findings

All animals were submitted to a complete necropsy. In all succumbed piglets (n=31), pathomorphological examination revealed most of the following lesions: haemorrhagic infarcts of the spleen, petechial haemorrhages in the kidneys, urinary bladder and lymph nodes, mucosal membranes and serous membranes. Pathomorphological examination indicated petechial haemorrhages in most organ systems and serous membranes (hemorrhagic diathesis) that are considered an important characteristics of CSF (Trautwein, 1988; van Oirschot and Terpstra, 1989). In the control group pathomorphological lesions were characteristic for the acute course of CSF, what is in accordance with the results of Milanov *et al.* (2002).

In a 28 days old piglets that survived contact infection, pathomorphological examination revealed changes characteristic for CSF (petechial haemorrhages in lymph nodes and infarction of the spleen). Moreover, a fibrinous and diphteroid inflammatory reaction was present in the digestive tract. Also, in 3 piglets 54 days old that survived experimetal infection, pathomorphological examination detected changes that suggest a successful infection with CSFV (petechial haemorrhages in lymph nodes). Although, before euthanasia of surviving animals no clinical signs typical for CSF were observed. The clinical protection from CSF is not the primary aim in pigs, and it might even be contraindicated since it can mask a present infection in an animal that may be a source of secondary outbreak of the disease (Dewulf, 2002).

Detection of CSFV antigen and viral RNA in blood samples

The results related to CSFV antigen (ELISA) and genome detection (RT-PCR technique) in blood samples according to groups, are summarized in Table 2. In blood samples of piglets that originate from vaccinated sows viraemia was detected by using one of the applied methods. In all i/m inoculated piglets (by RT–PCR technique) and in piglets from the control group (by ELISA) viraemia was detected already on 3 dpi. In blood samples of piglet No. IV/5 that survived challenge infection, only on 15 dpi doubtful reaction on presence of CSFV antigen (by ELISA test) was detected. Despite the fact that two piglets (No. IV/2 and IV/4) survived contact infection, in the period from 11 to 13 dpi a doubtful reaction was detected by ELISA test. By applying RT-PCR technique the presence of viral RNA was detected in blood samples. This finding is in accordance with the opinion that in piglets with maternal antibodies after infection, CSFV can multiply without

showing visible clinical signs (Terpstra, 1988). Consequently, such infected animals may act as a source of further dissemination of the virus. Our results support the conclusion that piglets with maternal antibodies do not succumb when they get infected with virulent CSFV. Depner *et al.* (2000) concluded that the presence of maternal antibodies influences the clinical course of CSF in terms that the outcome is rather transient than lethal. Such animals could play a crucial role in spreading CSFV and might contribute to the maintenance of long lasting epizootics. In this experiment, positive results on the presence of CSFV in the circulaton may present sufficient evidence that surviving piglets may represent a source of infection.

In the piglets 28, 35, 44 and 54 days old, that were exposed to contact infection, viraemia was at the earliest detected by ELISA test on 7 and 9 dpi. Applying RT-PCR technique on blood samples two days earlier (from the first positive result by ELISA test) the presence of viral RNA was detected only in 3 piglets. In the piglet 28 days old that survived contact infection, the viral antigen was detected at the latest (13 dpi). Positive results were confirmed again on 23 and 27 dpi. A transient disappearance of CSFV from the blood corresponded with the clinical improvement, and by the end of the experiment this piglet recovered completely. On the contrary, in the piglet No. I/5 CSFV the antigen was not detected in blood samples during the trial and this piglet had succumbed the earliest in the group. After experimental infection of piglets Laevens *et al.* (1998) found that the latency period from inoculation to viraemia in the experimentally inoculated pig lasted 4 days, while piglets that were exposed to horizontal infection, the first viraemia was detected on 12 and 14 dpi. The different periods at which viraemia in piglets was established may be due to the fact that viraemia with a short duration may be missed when samples are taken every two days, or that viraemia remains under the detection limit (Dewulf, 2002). The negative results could be due to neutralization of the virus by antibodies in the bloodstream, thus underestimating the viral load (Uttenthal *et al.,* 2003). It can be concluded that in these piglets the maternal antibody status might have influenced the course of the disease.

Considering the results of examination of viraemia susceptible piglets in all groups, in animals infected by contact, viral RNA in blood samples was at the earliest detected on 9 dpi. Applying the ELISA test for examination, viraemia was detected later, i.e. the earliest on 11 dpi and in the most of animals on 13 dpi. This points out that RT-PCR technique detected viraemia in susceptible piglets 2 days prior to detection of viraemia by the use of ELISA test. Determination of viraemia is considered useful in early detection of CSF in a herd, since this can be done before fever or clinical signs are evident. It is also highly important that the number of samples have to be taken throughout the farm and should be focusing on young piglets in order to be sure to sample viraemic animals (Koenen and Lefebvre, 1994). Since it is difficult to recognize the disease clinically in its early stage, our findings confirm the results of Dewulf (2002) that RT-PCR technique detects viraemia in pigs, and that the beginning of the viraemic period highly corresponds with the period of fever.

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Table 2. Detection of CSFV antigen in blood samples of experimental animals by IDEXX ELISA (viraemia) and detection of viral RNA by RT-PCR technique

Group A	No.	Days post infection														
		0	3	5	7	9	11	13	15	17	19	21	23	25	27	29
i/m challenge	I/1	–	± ⊕	– Ø	+	†										
	I/6	–	+	+	+	†										
Contactly infected piglets	I/2	–	–	–	– Ø	– Ø	+	+	†							
	I/3	–	–	–	– Ø	– Ø	– Ø	+	– Ø	– Ø	– Ø	– Ø	+	– Ø	+	– Ø
	I/4	–	–	–	– Ø	± Ø	+	+	†							
	I/5	–	–	–	– Ø	– Ø	†									
Susceptible piglets	N/1	–	–	–	– Ø	– Ø	+	†								
	N/2	–	–	–	– Ø	± Ø	+	†								
Group B																
i/m challenge	II/2	–	+	+	†											
	II/3	–	–	+	+	+	†									
Contactly infected	II/1	–	–	– Ø	+	– Ø	+	+	†							
	II/4	–	–	– Ø	– Ø	+	±	†								
	II/5	–	–	– Ø	– Ø	+	†									
	II/6	–	–	–	– Ø	– Ø	+	†								
Susceptible piglets	N/3	–	–	–	– Ø	– ⊕	+	+	†							
	N/4	–	–	–	– Ø	– ⊕	+	+	†							

Cont Table 2

	No.	Days post infection														
		0	3	5	7	9	11	13	15	17	19	21	23	25	27	29
Group C																
i/m challenge	III/3	–	± ⊕	+	+	+	†									
	III/4	–	± ⊕	+	+	+	†									
Contactly infected	III/1	–	–	–	–	–	– ⊕	†								
	III/2	–	–	– ⊕	+	– Ø	†									
	III/5	–	–	–	±	–	– ⊕	+	±	+	±	+	†			
	III/6	–	–	–	–	– ⊕	+	†								
Susceptible piglets	N/5	–	–	–	–	– ⊕	+	+	±	†						
	N/6	–	–	–	–	– ⊕	+	+	±	±	†					
Group D																
i/m challenge	IV/3	–	– ⊕	±	+	†										
	IV/5	–	– ⊕	–	–	–	–	–	±	–	–	–	–	–	–	–
Contactly infected	IV/1	–	–	–	–	–	± ⊕	+	±	±	–	†				
	IV/2	–	–	–	–	–	± Ø	– ⊕	–	–	–	–	–	–	±	–
	IV/4	–	–	–	–	–	± Ø	± Ø	–	–	–	–	–	–	–	–
	IV/6	–	–	–	–	–	– ⊕	+	+	+	†					
Susceptible piglets	N/7	–	–	–	–	– ⊕	+	+	–	–	†					
	N/8	–	–	–	–	–	– ⊕	+	†							
Group E																
Susceptible piglets	K/1	–	+	+	+	+	+	†								
	K/2	–	+	+	+	+	+	†								
	K/3	–	+	+	+	+	†									

No. – ear-tag number of pigs; (–) negative ELISA result; (±) doubtful ELISA result; (+) positive ELISA result; (⊕) – positive RT-PCR result; (Ø) – negative RT-PCR result; † – death of experimental animal

Detection of CSFV in tissue samples

The results of CSFV antigen and genome detection in tissue samples are summarized in Table 3. In i/m infected piglets the viral antigen detection and distribution in tissues was uniform, i.e. in all the examined tissue samples deriving from inoculated piglets the presence of CSFV antigen was detected by the ELISA test. This result is in accordance with the data that highly virulent viruses can be detected in most of the organs 5 - 6 dpi (van Oirschot and Terpstra, 1989). There is an exeption from this finding in one survived piglet (No. IV/5). In the examined tissue samples deriving from this piglet CSFV antigen was not detected by ELISA test. However, after applying additional tests in the examined tissue samples, the presence of viral RNA was confirmed in tonsillar tissue and in the terminal part of the ileum using RT-PCR technique. By comparing the obtained results in directly infected piglets with the results of the ones infected by contact, at the same age period, differences can be observed. In the group of piglets exposed to horizontal infection, the largest number of positive results was detected in examined tissue samples of tonsils and terminal ileum (82%), than in the spleen (75%) and mandibular lymph nodes (50%), while the smallest number of positive samples was detected in the examined kidney tissue (31%).

In tissue samples deriving from piglets that survived horizontal infection CSFV antigen was not detected by ELISA test. By applying additional testing, the presence of viral RNA was confirmed in samples of tonsils and terminal part of ileum (No. IV/2 and IV/4) and in all examined tissue samples deriving from piglet No. I/3 by RT-PCR technique. In the most of the examined tissue samples derived from susceptible piglets, and in all the examined tissue samples derived from the control group, the presence of CSFV antigen was detected by ELISA. It is considered that in the case of chronic and subclinical infection, CSFV antigen may be detected in epithelial cells of tonsils, in the ileum and lymph nodes. The spleen and ileum being the most appropriate tissues for sampling for early detection of the virus (De Smit, 2000). It is especially emphasized that the ileum is frequently found to be positive in more prolonged cases of CSF infections (van Oirschot and Terpstra, 1989). The obtained results are in agreement with the opinion that the tonsils are not only the primary target organ for CSFV multiplication, but also the site for CSFV persistence. CSF virus may persists for a long time in the tonsils independently, whether the animals have antibodies or not (Depner *et al.,* 2000). The surviving piglets were clinically but not virologically protected, and it may be considered that secondary outbreaks are not prevented as long as sources of infection remain present (Dewulf, 2002). Because subclinically infected pigs may live for months and shed the virus, they represent a major impediment to the control of epizootics (Kleiboeker, 2002). The obtained results suggest that not all piglets born to vaccinated sows have maternal antibodies at a detectable level, and the question of the efficiency of passive immunization need to be further evaluated in the future.

Table 3. Detection of CSFV in tissue samples of experimentally infected piglets

	No.	Spleen	Kidney	Mandibular lymph nodes	Tonsils	Ileum
Group A						
i/m challenged	I/1	+	+	+	+	+
	I/6	+	+	+	+	+
Contactly infected	I/2	+	+	+	+	+
	I/3	– | ⊕	– | ⊕	– | ⊕	– | ⊕	– | ⊕
	I/4	+	+	+	+	+
	I/5	+	–	±	+	+
Susceptible piglets	N/1	+	±	+	+	+
	N/2	±	-	+	+	+
Group B						
i/m challenged	II/2	+	+	+	+	+
	II/3	+	+	+	+	+
Contactly infected	II/1	+	±	+	+	+
	II/4	+	–	+	+	+
	II/5	+	–	–	+	+
	II/6	+	–	–	+	+
Susceptible piglets	N/3	+	+	+	+	+
	N/4	+	+	+	+	+
Group C						
i/m challenged	III/3	+	+	+	+	+
	III/4	+	+	+	+	+
Contactly infected	III/1	+	±	+	+	+
	III/2	–	–	±	+	+
	III/5	+	+	+	+	+
	III/6	+	–	–	+	+
Susceptible piglets	N/5	+	+	+	+	+
	N/6	+	+	+	+	+
Group D						
i/m challenged	IV/3	+	+	+	+	+
	IV/5	– | Ø	– | Ø	– | Ø	– | c	– | ⊕
Contactly infected	IV/1	+	+	+	+	+
	IV/2	– | Ø	– | Ø	– | Ø	– | ⊕	– | ⊕
	IV/4	– | Ø	– | Ø	– | Ø	– | ⊕	– | ⊕
	IV/6	+	+	+	+	+
Susceptible piglets	N/7	+	±	+	+	+
	N/8	+	–	+	+	+
Group E						
i/m infected	K/1	+	+	+	+	+
	K/2	+	+	+	+	+
	K/3	+	+	+	+	+

No. – ear-tag number of pigs; (+) positive ELISA result; (–) negative ELISA result; (±) doubtful ELISA result; (⊕) – positive RT-PCR result; (Ø) – negative RT-PCR result

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ISPITIVANJE PASIVNOG IMUNITETA KOD PRASADI POREKLOM OD KRMAČA VAKCINISANIH KINA SOJEM VIRUSA KLASIČNE KUGE SVINJA

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SADRŽAJ

Ova istraživanja su izvršena u cilju praćenja toka oboljanja nakon infekcije prasadi virusom klasične kuge svinja (KKS) poreklom od krmača vakcinisanih Kina sojem virusa KKS. Ogled je obavljen na 24 praseta (uzrasta 28, 35, 44 i 54 dana) poreklom od vakcinisanih krmača i na 11 nevakcinisane prasadi poreklom od nevakcinisanih krmača. Po dva praseta iz svake starosne grupe, poreklom od vakcinisanih krmača, su veštački inficirana intramuskularnom aplikacijom virusa KKS. U cilju ustanovljavanja kontaktne (horizontalne) infekcije, direktno inficiranim jedinkama su dodata po četiri praseta istog uzrasta poreklom od vakcinisanih krmača i dva praseta poreklom od nevakcinisanih krmača. Nakon veštačke infekcije, vršen je klinički pregled i uzorkovanje krvi od svake životinje 0, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27 i 29 dana ogleda. Uzorci krvi su ispitivani ELISA testom na prisustvo specifičnih antitela protiv virusa KKS, na prisutvo antigena virusa KKS odnosno virusne RNA metodom RT-PCR. Nakon uginuća ili žrtvovanja, vršen je patomorfološki pregled i utvrđivanje prisustva i distribucije antigena virusa u uzorcima tkiva ELISA testom. Na osnovu postignutih rezultata istraživanja može se zaključiti da pojedina prasad, poreklom od vakcinisanih krmača, nemaju detektabilna specifična antitela što nameće razmatranje pitanja efikasnosti pasivne imunizacije.